

# **Delineating Thyroid-Mediated Toxicity Pathways in an Amphibian Model System**

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# Outline

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- Brief background on MED thyroid research
- Approach to adverse outcome pathway research for amphibian-based thyroid model
- Investigating endpoints at steps in the pathway
- Understanding toxicity in the context of pathway information



# Background on EDC Assays

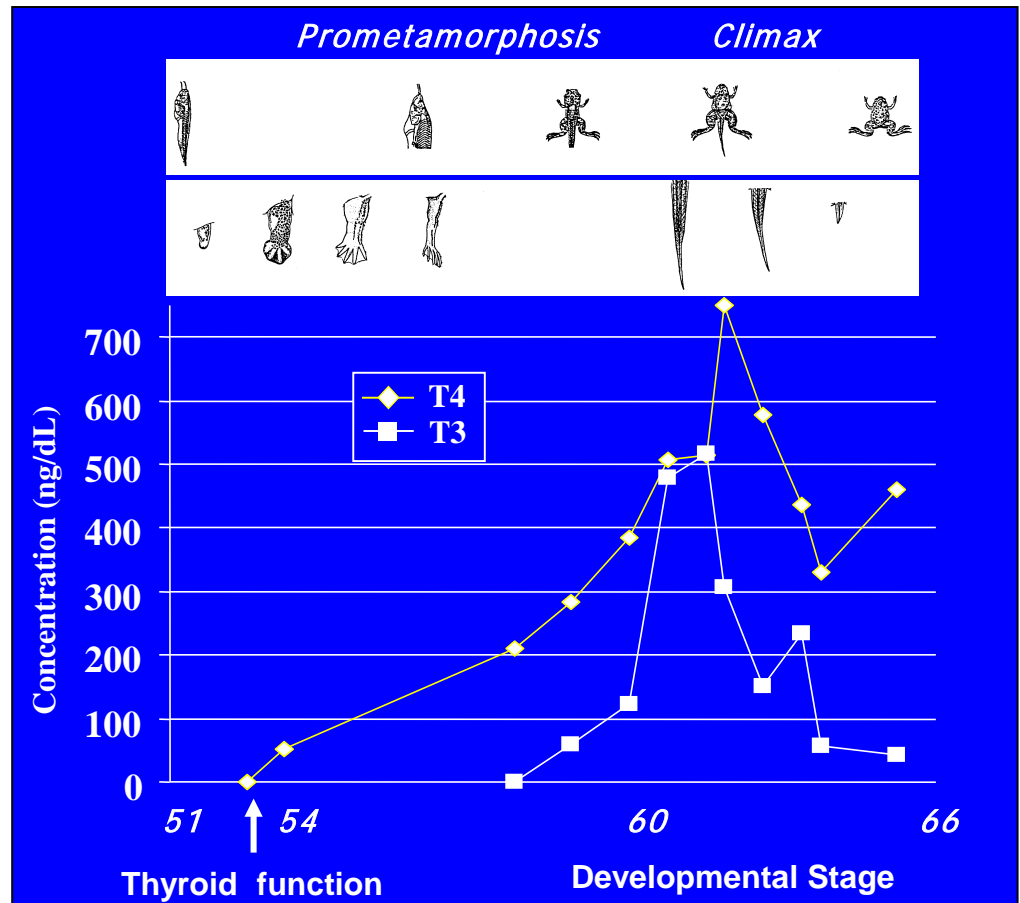
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- FQPA and SDWA directed U.S. EPA to evaluate chemicals for endocrine disruption
- Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) established
- Screening and testing methods recommended by EDSTAC included:
  - Short-term fish reproduction assay to detect HPG effects
  - **Short-term amphibian metamorphosis assay to detect HPT effects**
  - Longer-term fish life-cycle assay for reproductive effects
  - Longer-term amphibian reproductive/developmental effects



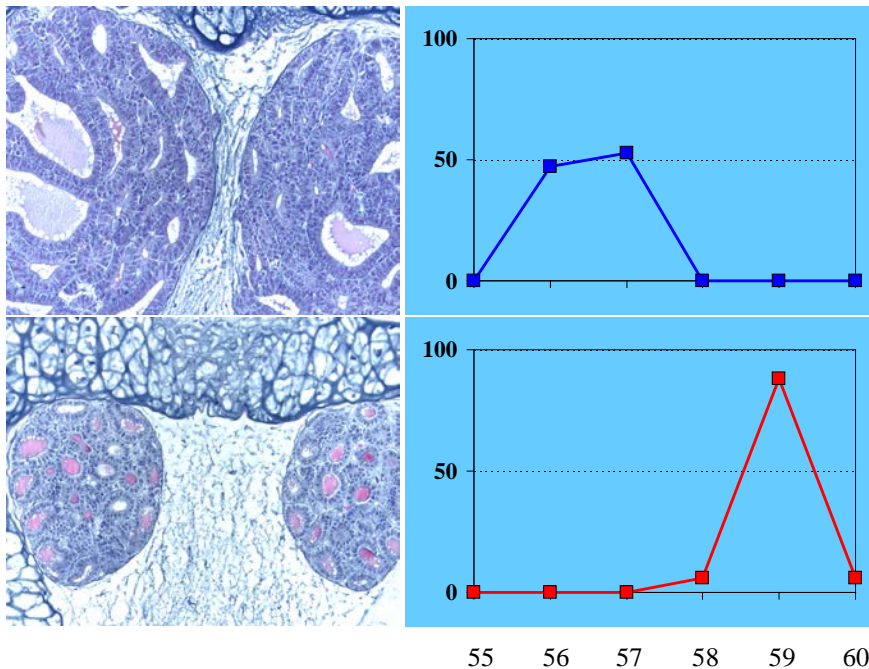
# Test Overview

- Initiated with tadpoles at onset of thyroid function
- 14-21 days exposure
- Apical Endpoints
  - Developmental stage
  - Thyroid histology

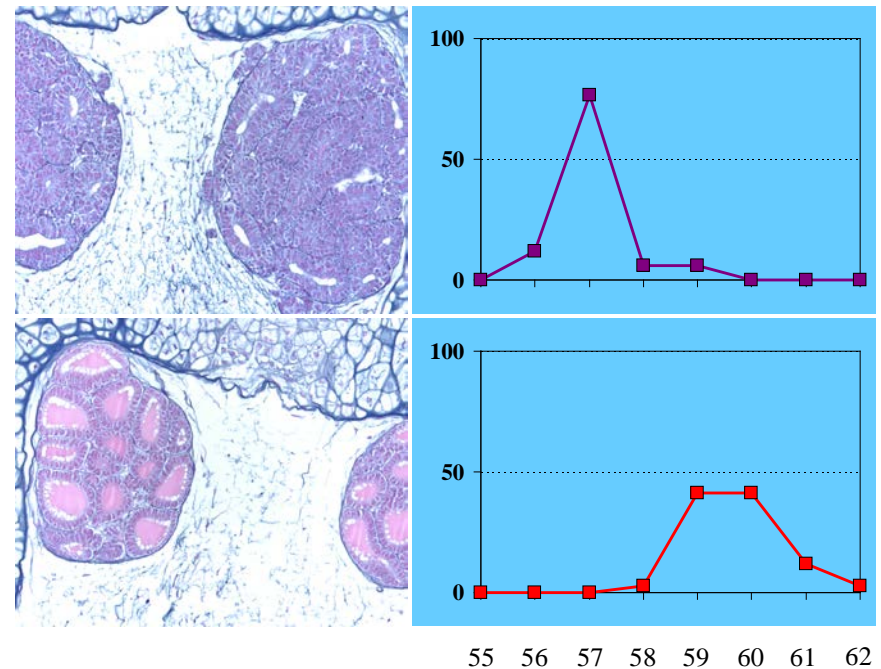


# Amphibian Metamorphosis Assay

## Methimazole

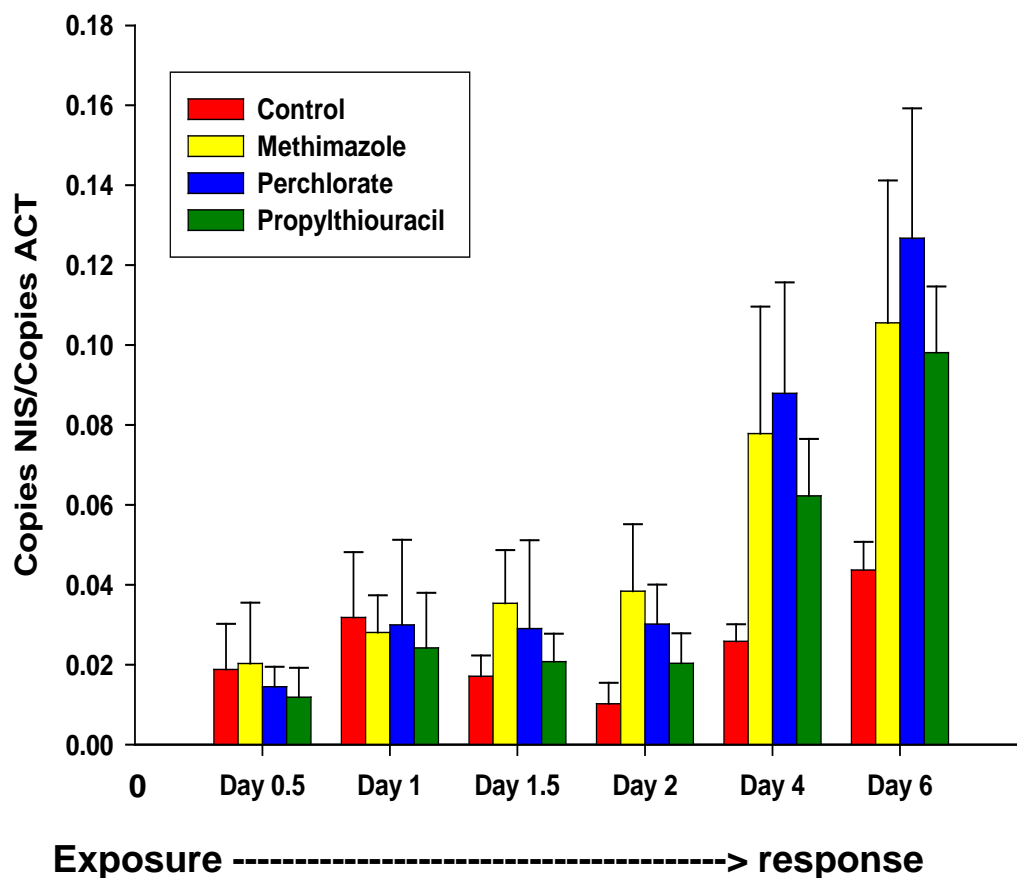


## Perchlorate



# In Vivo Gene Expression Following Exposure

## NIS Gene Response





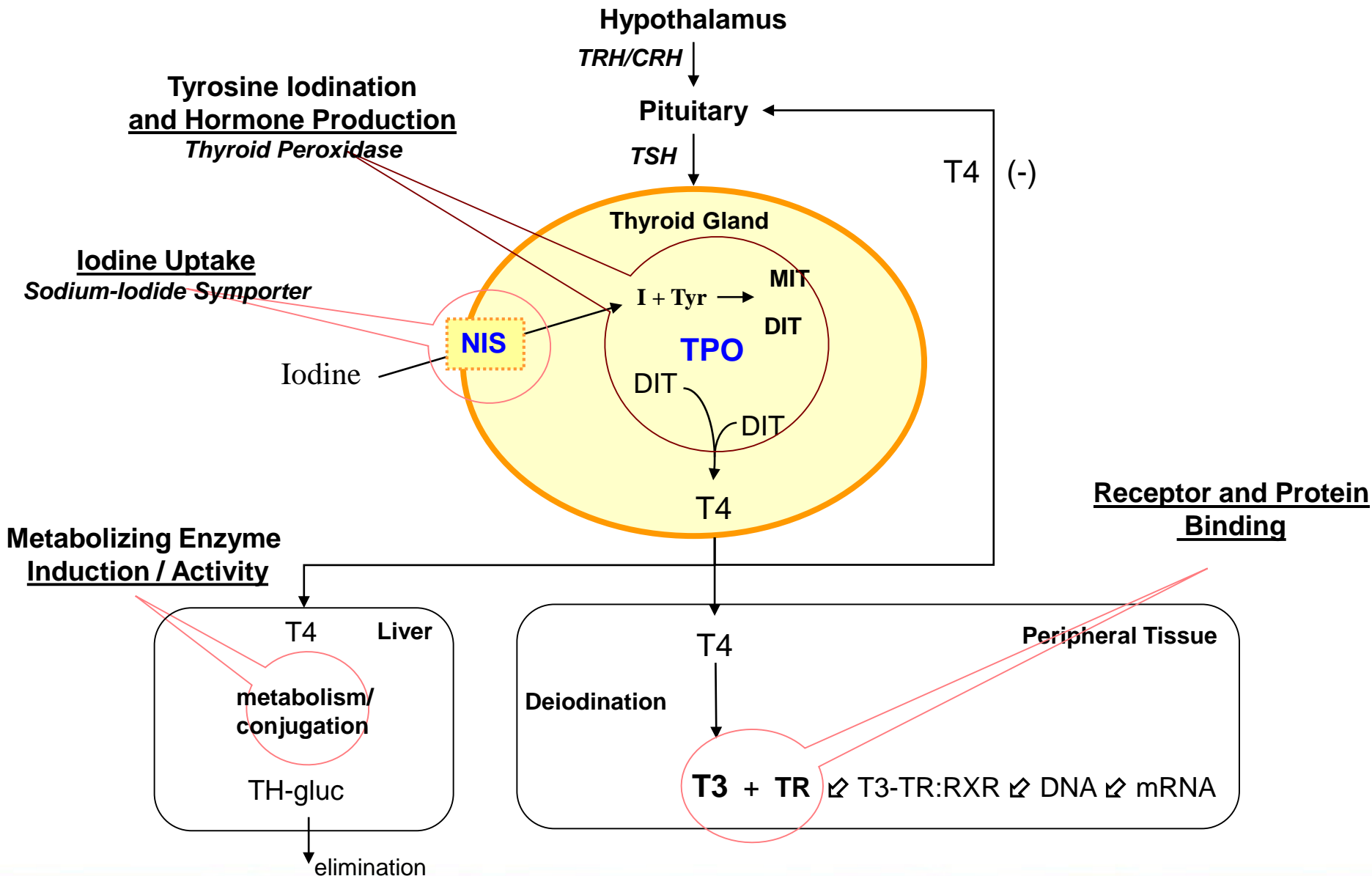
# The Need for Predictive Tools

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- Apical endpoints established with the amphibian metamorphosis assay.
- However we can not test all the chemicals for which little is known about thyroid activity
- Need to develop tools to aid in selecting chemicals that need to be tested and inform which chemicals are likely not active and do not need to be tested or can be assigned low priority for testing

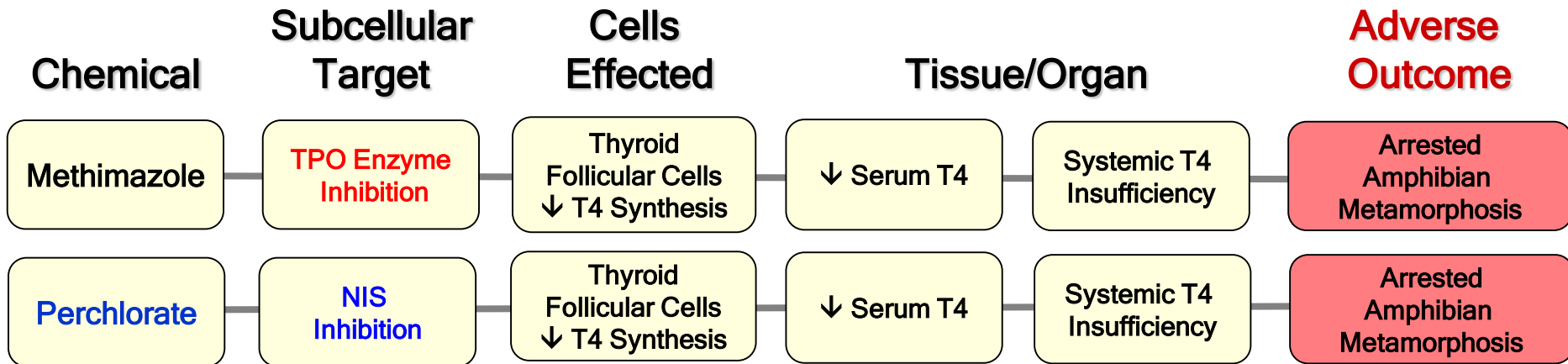


# Potential Endpoints for Thyroid Hormone Disruption





# Adverse Outcome Pathways for Thyroid Disruption



Toxicity Pathway

Adverse Outcomes Pathway

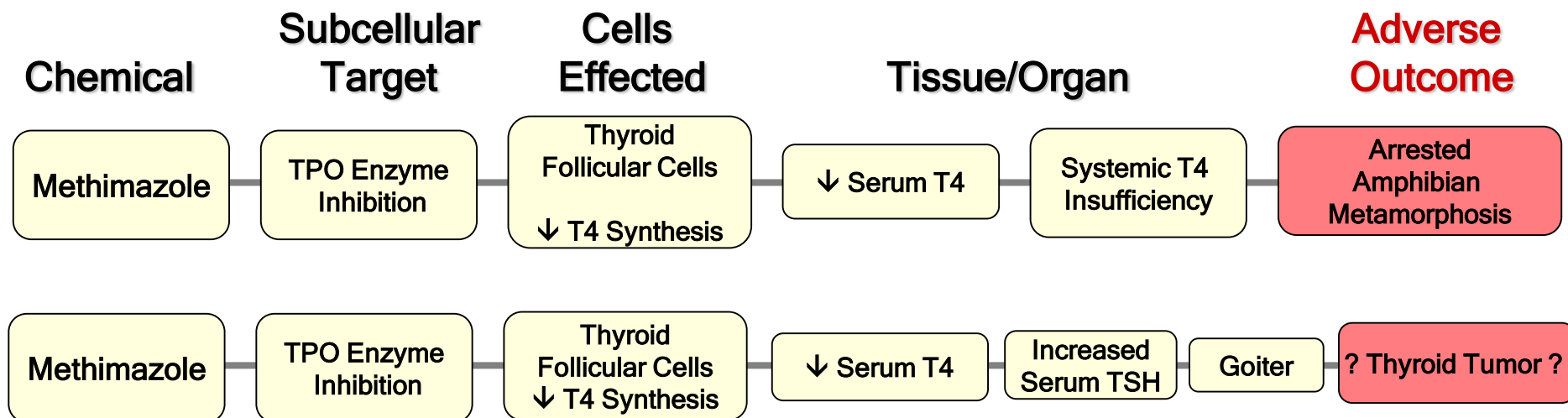
Amphibian Metamorphosis Assay



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# Selection of a Pathway



TPO-inhibition has potentially more types of chemicals that can affect it than NIS. Assays available.

Single chemical with potential for multiple adverse outcome pathways related to thyroid



# **Thyroid Explant Cultures and In Vitro Assays to Understand Responses Outside of the Compensatory Mechanisms of the Whole Animal**

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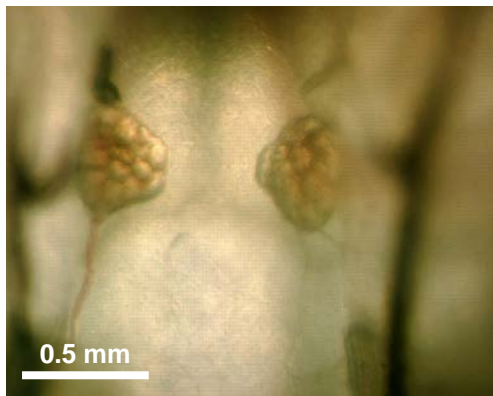
# Thyroid Gland Explant Cultures

## Ex Vivo:

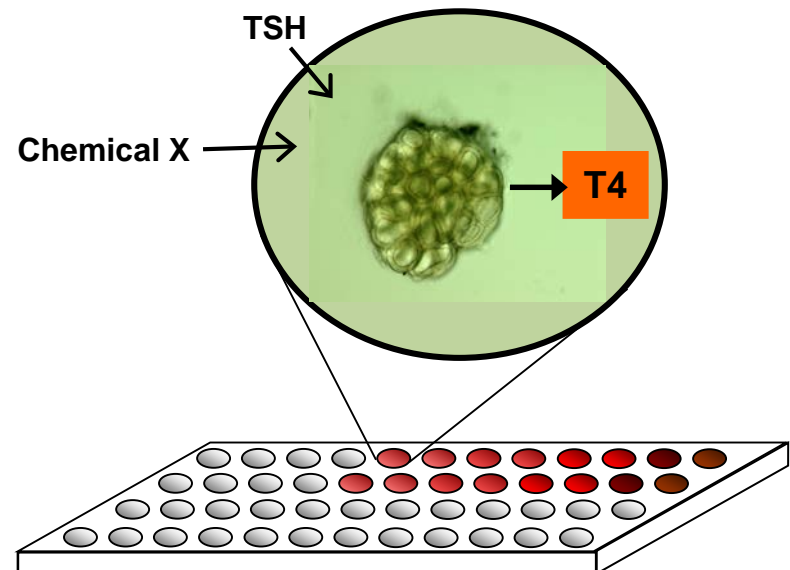
### Thyroid Gland Explant Cultures

Dissect thyroid glands from pro-metamorphic tadpoles

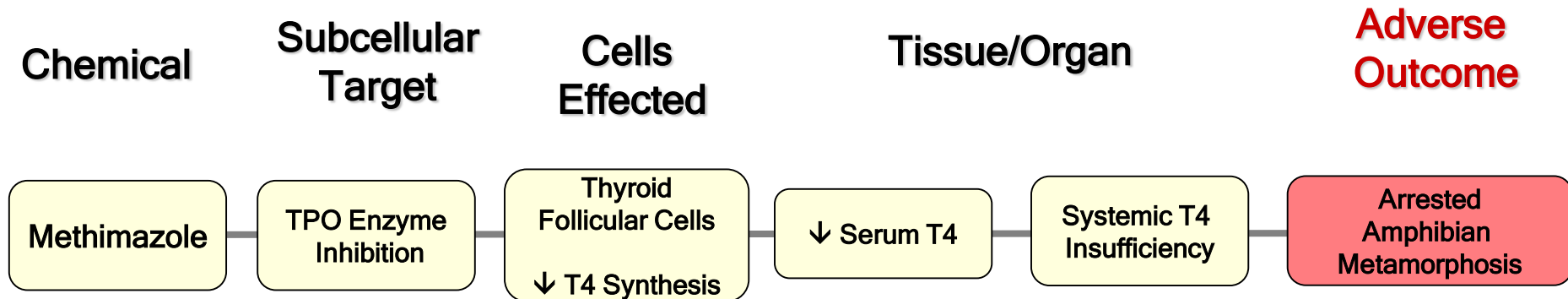
- Culture in 96-well plates in L-15 media
- Stimulate with TSH and treat with graded concentrations of test chemical
- Measure T4 released to media by RIA
- Measure TSH responsive gene expression by QPCR.



Paired thyroid glands in  
NF stage 59 *X. laevis* tadpole

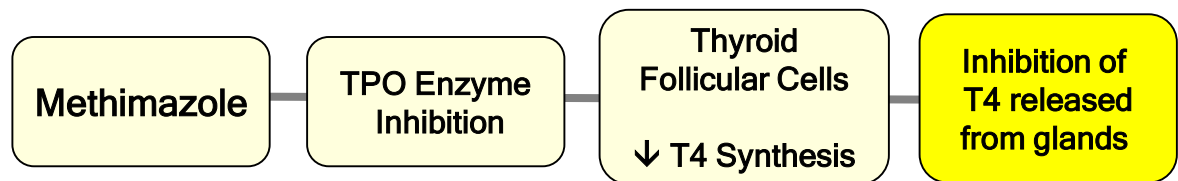


# Adverse Outcomes Pathway for Thyroid Toxicity



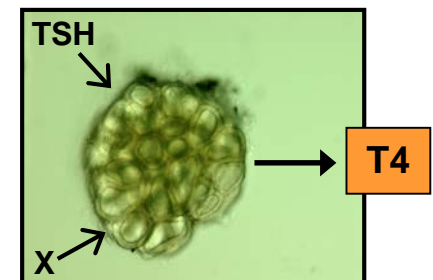
Toxicity Pathway

Adverse Outcomes Pathway



Thyroid Explant Culture Assay

Verification of chemical activity at organ level

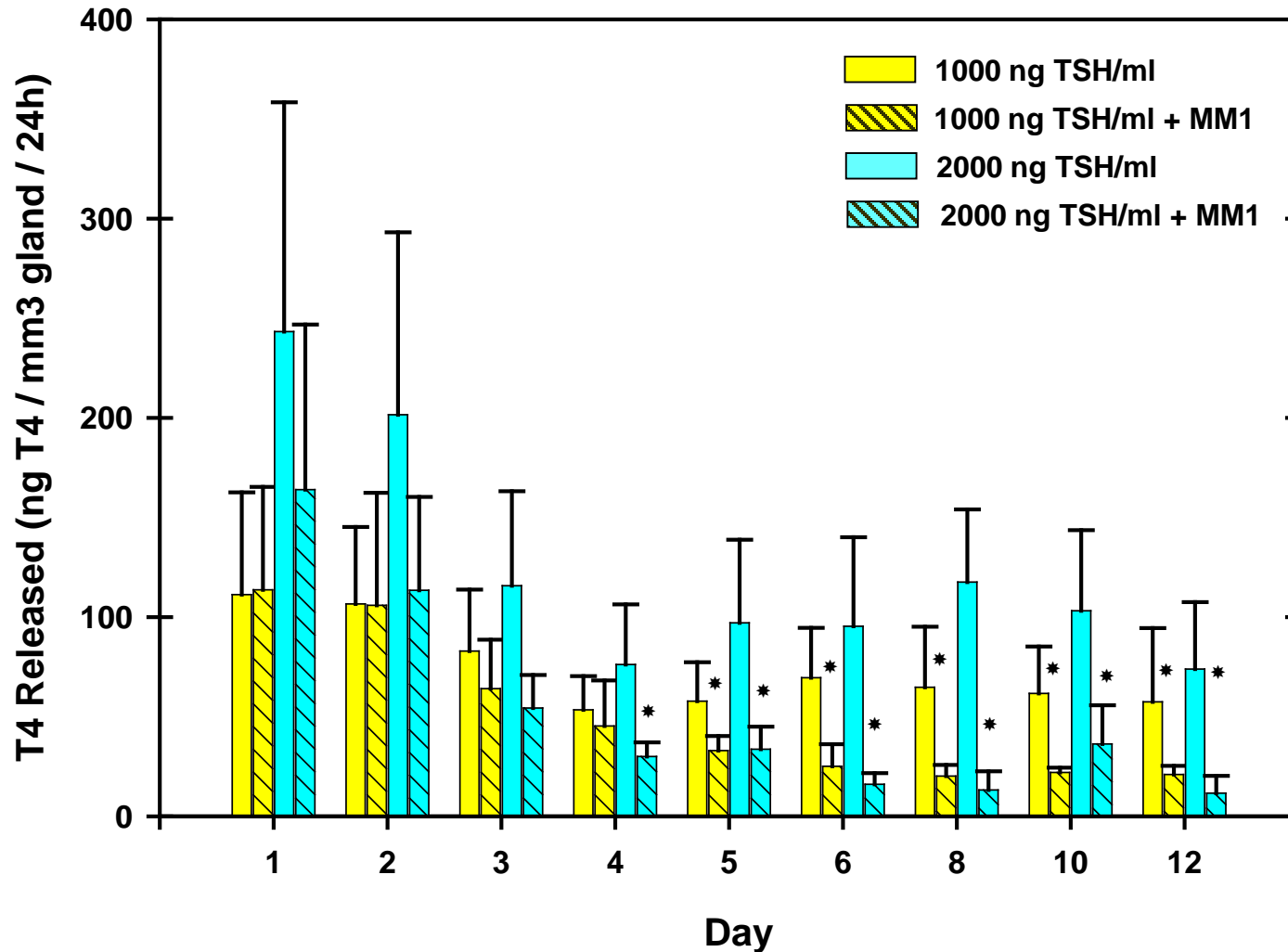


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# Thyroid Gland Explant Culture:

Time & dose relationship of T4 release inhibition



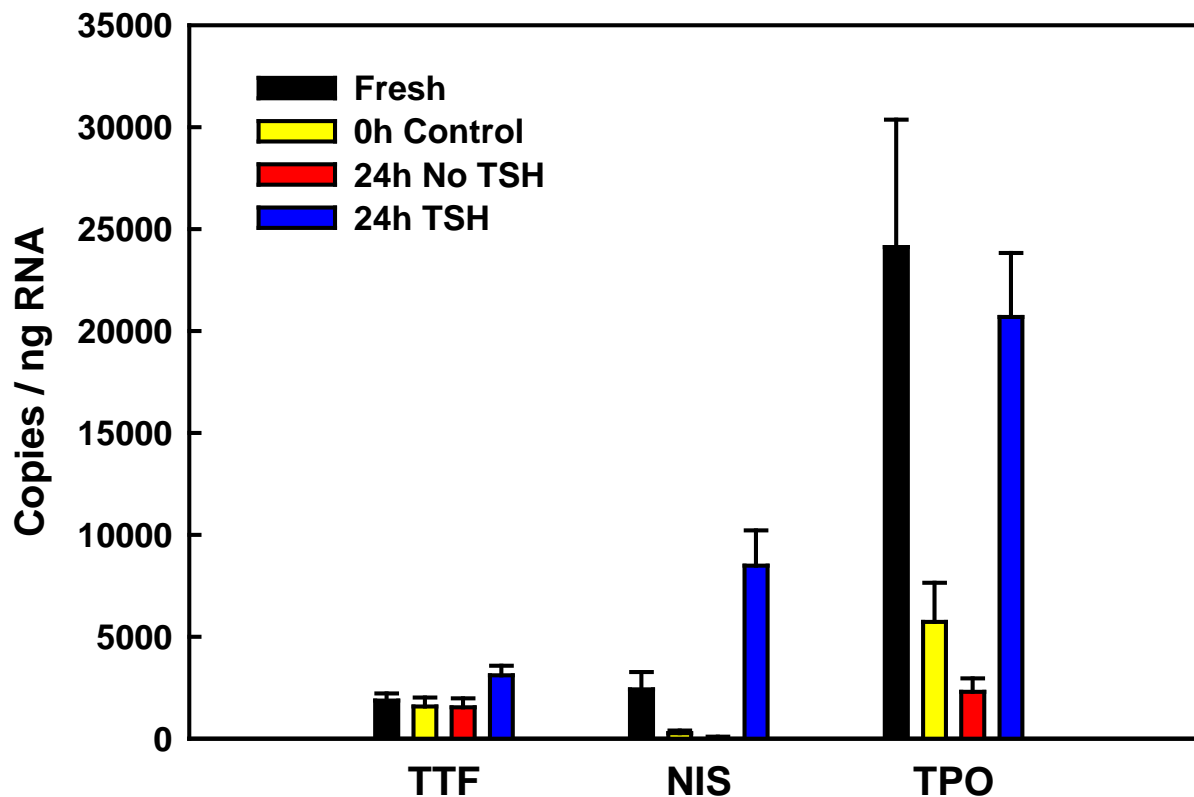
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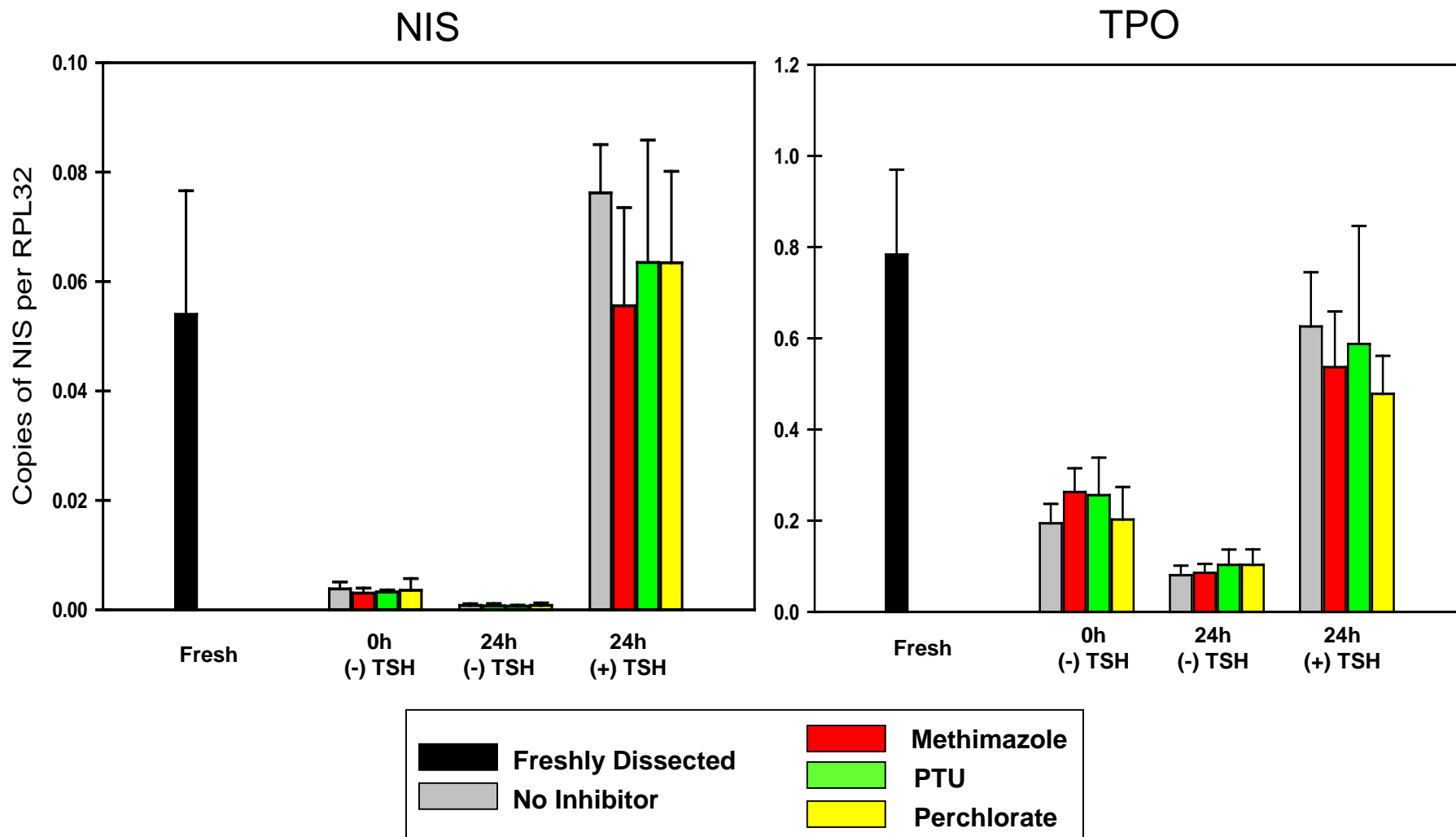
# Gene Expression in Thyroid Gland Explant Cultures

## Gene Expression in Response to TSH TTF, NIS, and TPO



# Thyroid Gene Expression: Response to Inhibitors

Methimazole, PTU, and Perchlorate Treatment



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# Thyroid Explant Culture

## Interpretation of compensatory and direct effects

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### *In vitro...*

- Release T4 in response to TSH is dose related
- T4 reserves must be depleted before synthesis inhibition significantly affects T4 release

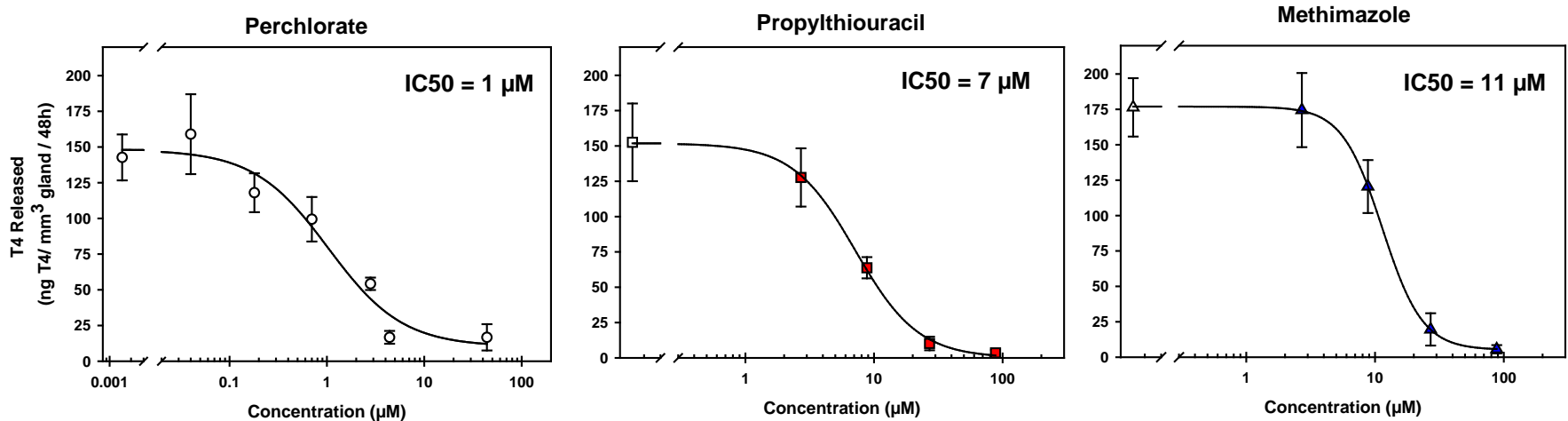
### *In vivo...*

- Early stages are more sensitive to arrested metamorphosis by T4 inhibitors than late stages
- At late prometamorphosis, thyroid glands are larger and reserve T4 is sufficient to complete metamorphosis
- Exposure time 0 does not equal effect time 0 for circulating T4: NEED TO UNDERSTAND DOSIMETRY / PK & PD
- Need to measure circulating hormone levels (T4, TSH) to interpret gene expression and protein responses in vivo



# Inhibition of TSH-Stimulated T4 Release by Cultured Thyroid Glands

Thyroid glands from *X. laevis* tadpoles (NF stage 59) were cultured in L-15 media in the presence of 2000 ng TSH/ml alone or TSH and graded concentrations of chemical. Media was collected and analyzed by RIA for T4.

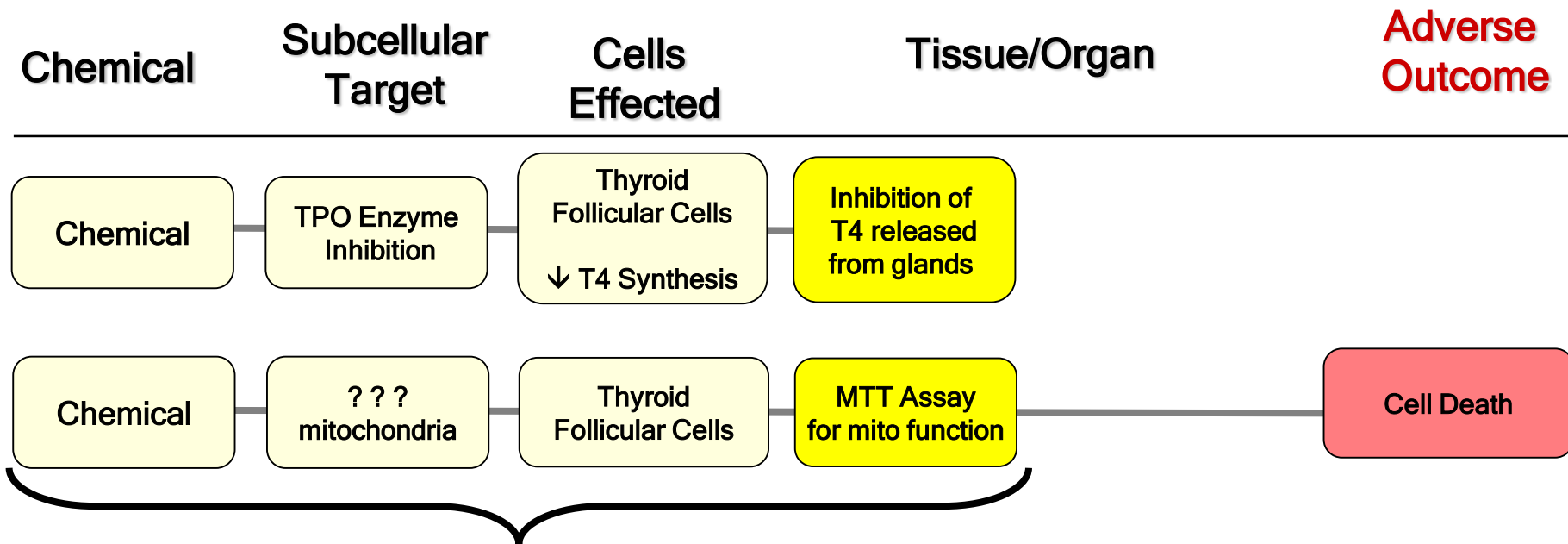


Perchlorate – Iodine uptake inhibition  
PTU & Methimazole – TPO inhibition

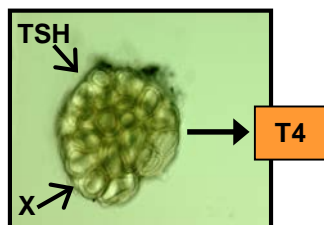
Understand results in the context of the assay



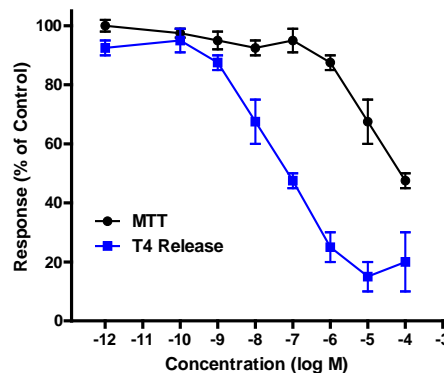
# Assess Pathway for Thyroid Disruption vs Thyroid Follicular Cell Toxicity



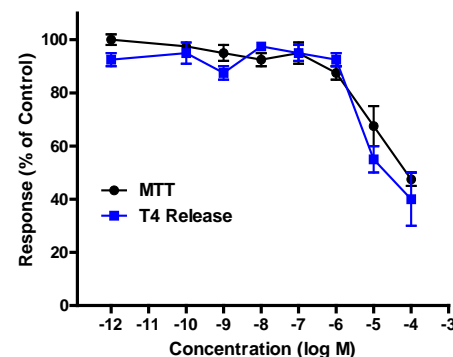
## Thyroid Explant Culture Assay



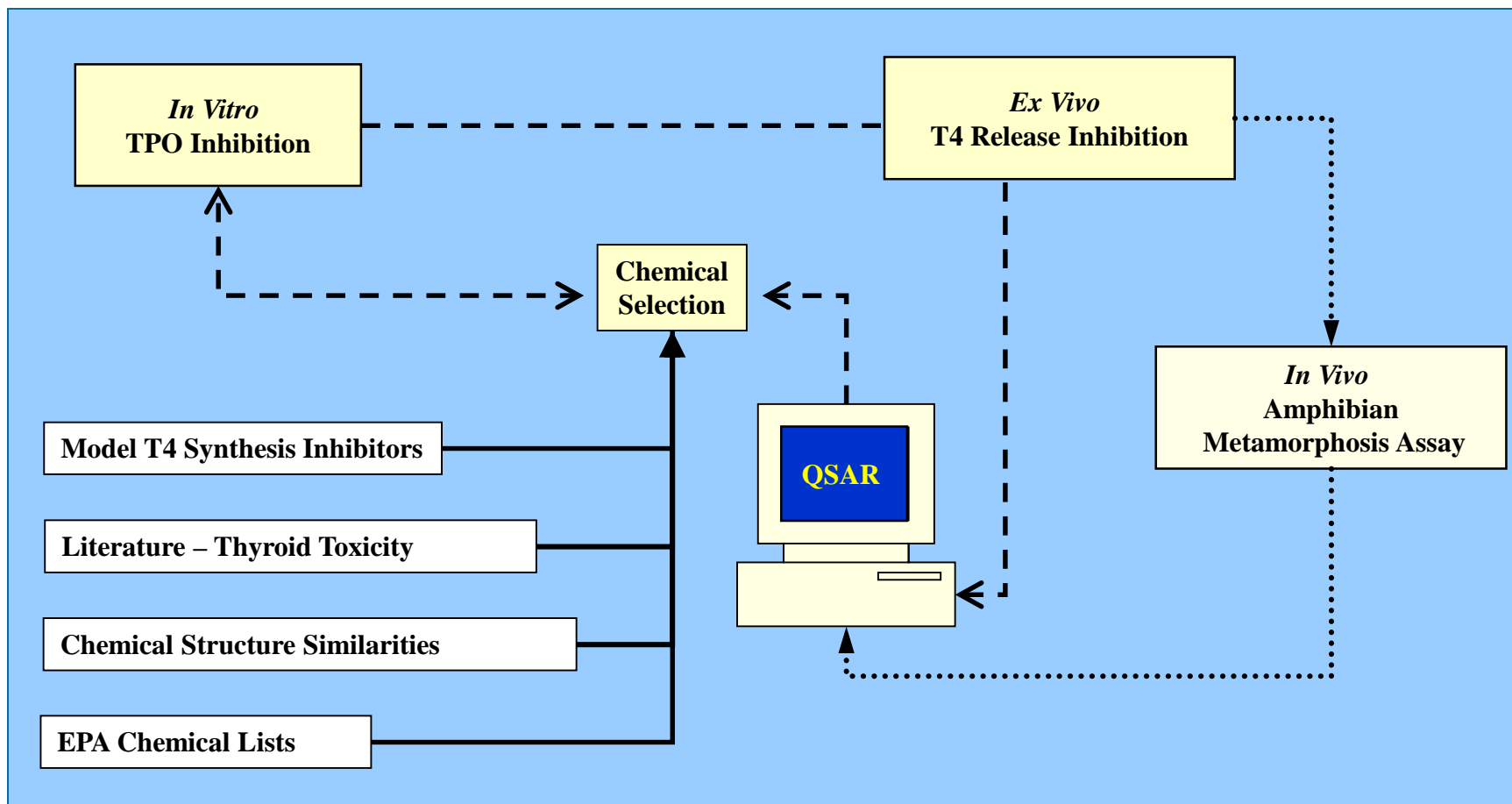
Thyroid Disrupting Chemical



Thyroid Toxic Chemical

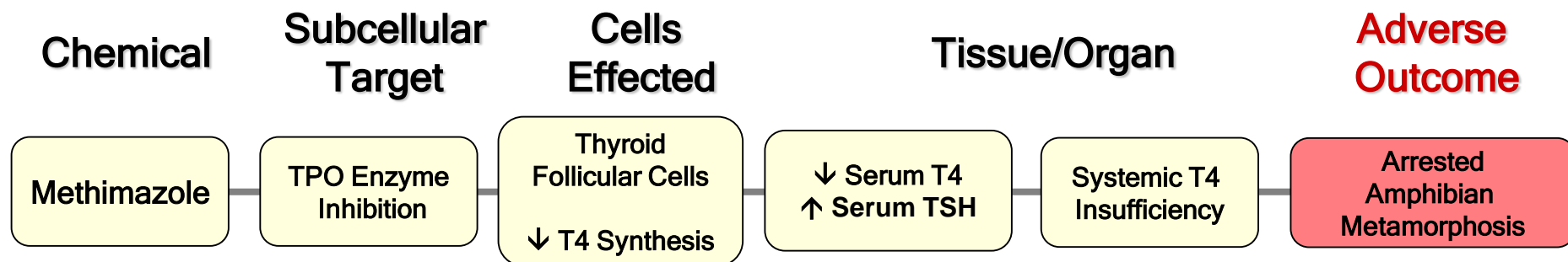


# Chemical Testing and Predictive Model Development

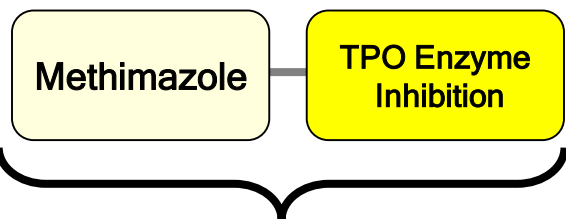




# Adverse Outcomes Pathway for Thyroid Toxicity

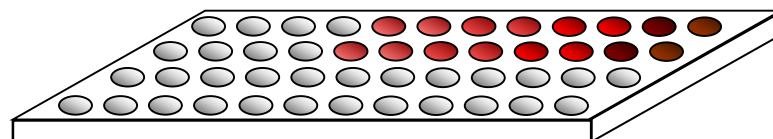


Toxicity Pathway



In Vitro TPO Inhibition Assay

QSAR = Chemical + Cellular Target



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# Thyroid Peroxidase Inhibition

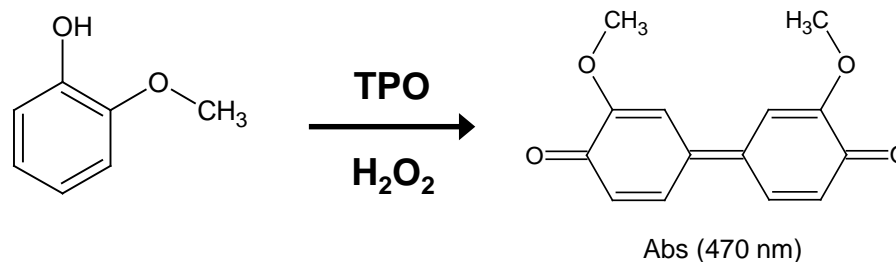
## In Vitro:

Prepare microsomes containing thyroid peroxidase activity from pig thyroid glands

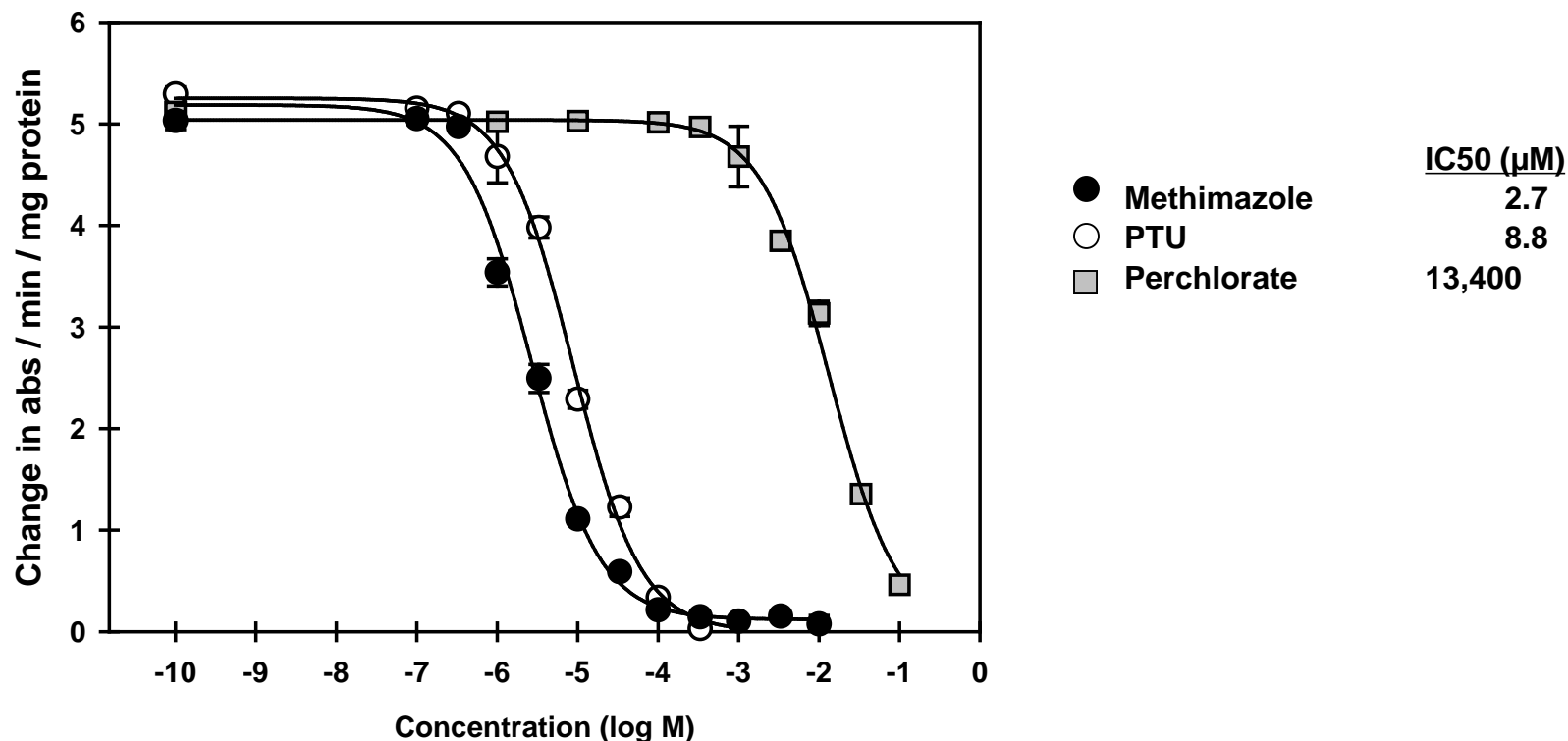
Test chemicals for potency for inhibiting two TPO-mediated reactions

1. Tyrosine Iodination: Conversion of Tyrosine to MIT/DIT
2. Guaiacol Oxidation: Surrogate coupling reaction ( $\text{DIT} + \text{DIT} = \text{T4}$ )

### Guaiacol Oxidation Assay



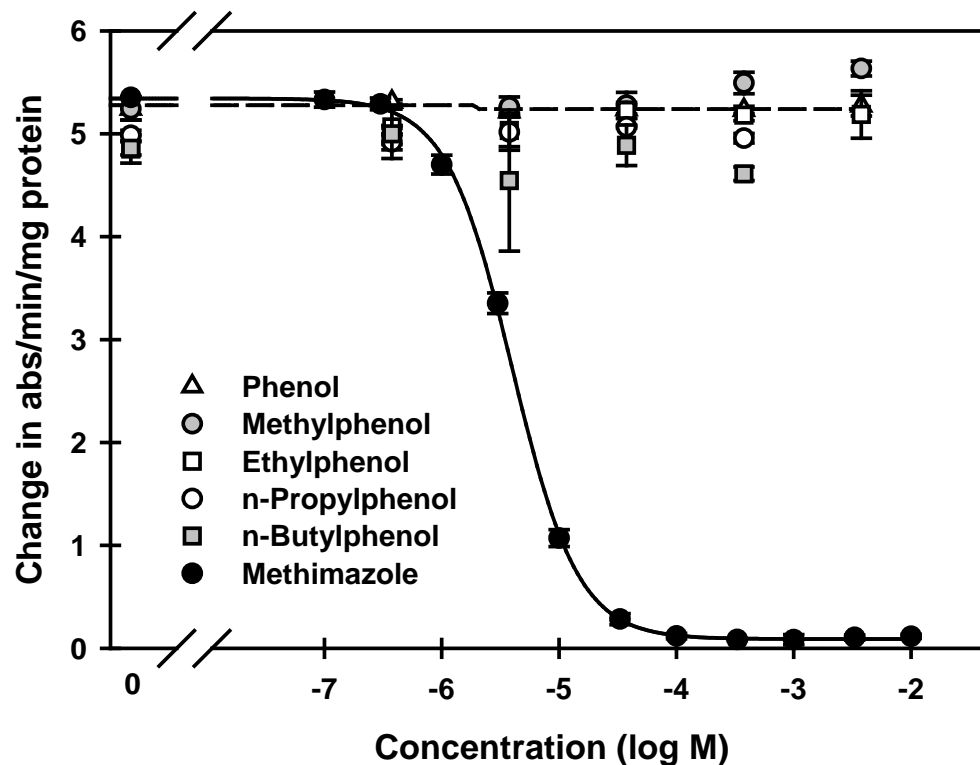
# Relative Potency of Model T4 Synthesis Inhibitors for TPO Inhibition



Methimazole is the most potent of the model T4 synthesis inhibitors in the TPO inhibition assay. Perchlorate had very low potency for TPO inhibition, but was the most potent of the three inhibitors in the *ex vivo* and *in vivo* assays. The primary mechanism of action for perchlorate inhibition of T4 synthesis is by inhibiting iodide uptake into the follicular cells.



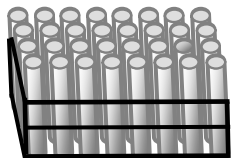
# TPO Inhibition: Alkylphenol Series



All alkylphenols tested exhibited no inhibition of TPO activity



# Screening Chemicals for Higher Tier Testing



**24 chemicals tested in vitro in TPO Inhibition Assay**

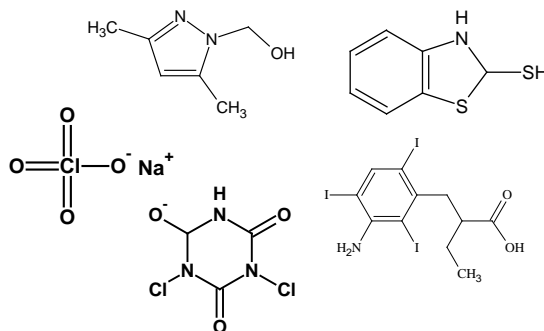
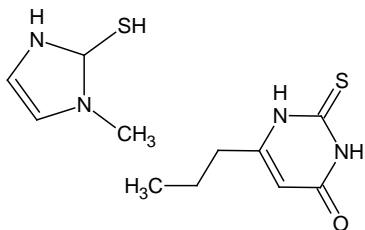
Active

Inactive

**7 Inhibit TPO In Vitro**

**17 Did Not Inhibit TPO**

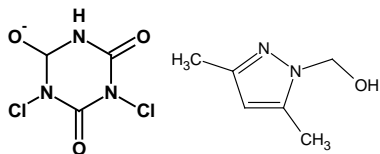
**Model TPO Inhibitors**



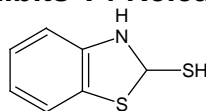
- Alkylphenol series
- Phthalates
- Isothiazoline
- Mixed Iodo Phenyl
- OH-PCDE
- Triazole
- Triazines
- Conazole

**Thyroid Gland Explant Culture: T4 Release**

**Cytotoxic / Negative**



**Positive:  
Inhibits T4 Release**



**Test In Vivo  
Amphibian  
Metamorphosis  
Assay**



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# TPO Assay Summary

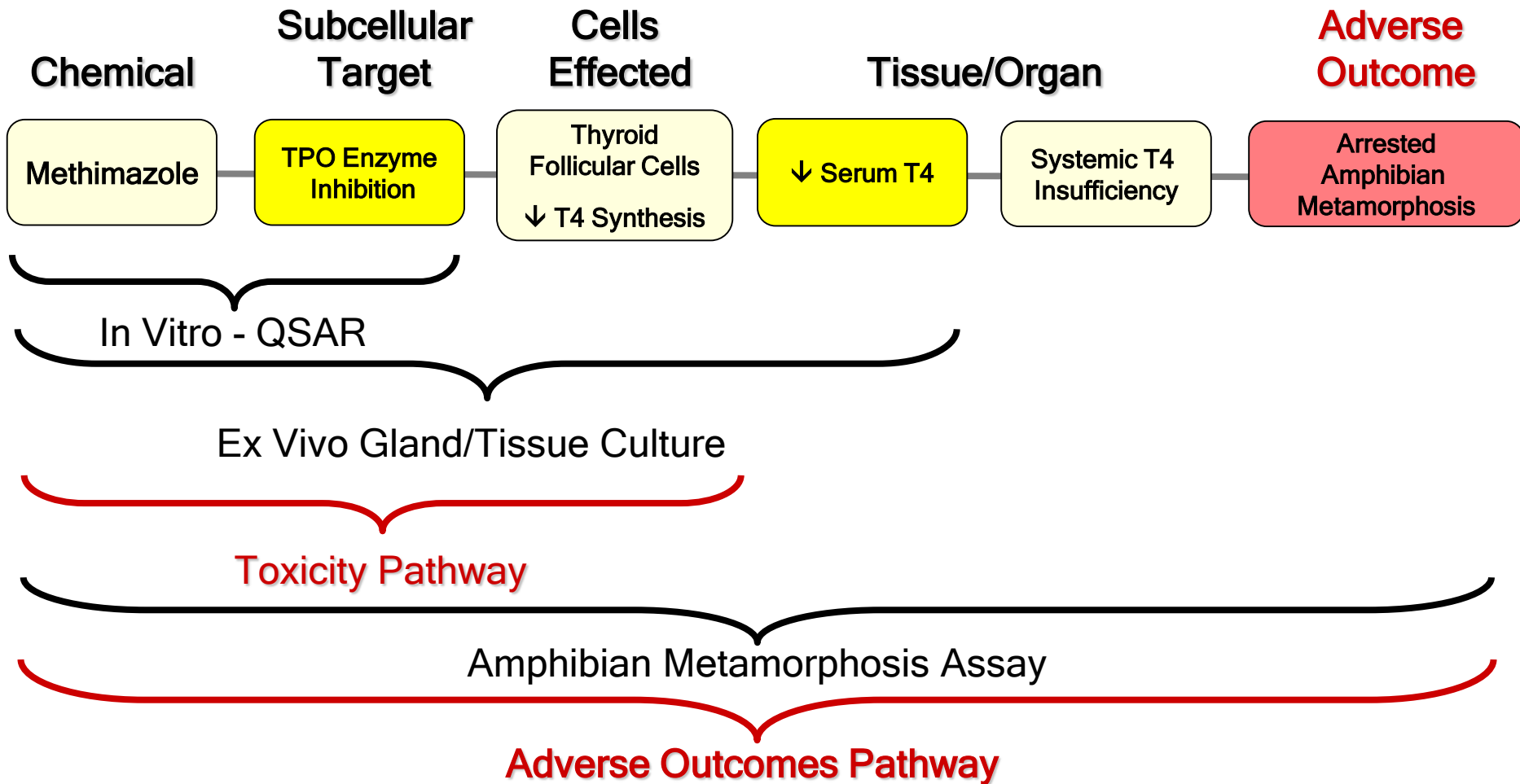
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- The TPO assay can be used to rapidly screen chemicals for further testing in the higher level thyroid toxicity assays, and can be used to begin to develop predictive models incorporating structure activity relationships between chemical structure and T4 synthesis inhibition
- This suite of assays can be an effective tool to determine the capacity of previously untested or unsuspected classes of chemicals to disrupt normal thyroid hormone production





# Adverse Outcomes Pathways for Thyroid Disruption



# MED Thyroid Project Team

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**Emily Burgess**

**Scott Moen**

